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(54) Human H5P 90 Epitopes

(57) There are disclosed functional epitopes which are purified from human HSP 90 or which are synthesised to correspond to such purified epitopes, which are, if purified, unchanged or changed by substitution of selected amino acids and if synthesised are identical to the purified epitopes or differ from the purified epitopes by substitution of selected amino acids, and which cross-react with an antibody raised against a stress protein. The stress protein may comprise a fungal stress protein e.g. Candida 92 and/or 47 kD; Aspergillus 40, 51 and/or 88/84 kD, Pneumocystis carnii proteins or a bacterial stress protein e.g. Streptoccal 90 kD; Coryneform 86 and/or 52 kD stress proteins.

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STRESS PROTEIN EPITOPES

This invention concerns stress protein epitopes intended inter alia for use in diagnosis and treatment of disease states in which stress proteins are produced.

Environmental stress can induce an increase in the rate of synthesis of so-called heat shock, or stress, proteins in both procaryotic and eucaryotic cells (see for example Schlesinger et al (eds) in: Heat Shock from Bacteria to Man, Cold Spring Harbor, New York, (1972)). Although the function of the stress proteins has yet to be finally resolved, some have been reported to participate in assembly and structural stabilisation of certain cellular and viral proteins, and their presence at high concentration may have an additional stabilising effect during exposure to adverse conditions.

Many pathogenic organisms have been shown to produce stress proteins (see Young et al, Proc. Natl. Acad. Sci. USA, 85, 4267-4270 (1988)). The proteins are thought to be produced in response to the stress of infection to help protect the invading pathogen. Thus, for example, the ability to produce stress proteins has been implicated in the survival of bacterial pathogens within macrophages (Christmas et al, Cell, 41, 753-762

(1985) and Morgan et al, Proc. Natl. Acad. Sci. USA, 83, 8059-8063 (1986)).

Burnie et al, (GB 90307236.1, WO 92/01717), have found that stress proteins from both fungi and bacteria, for example, Candida albicans and Corynebacterium jeikium, comprise an immunodominant conserved antigen. The carboxy end of the Candidal stress protein has been sequenced and an antibody raised against the epitope LKVIRKNIVKKMIE found to recognise both the 47 and 92Kd Candidal stress proteins in sera from patients suffering from systemic Candidal infection. In addition, the antibodies also recognised stress proteins in sera of patients suffering from other fungal infection, for example, the 40 and 88/84 Kd Aspergillus stress proteins, as well as stress proteins in sera from patients suffering from bacterial infection, for example, the 52 and 86Kd Coryneform stress proteins. Other peptide sequences from the Candidal stress protein were found to be immunogenic, for example, the epitopes LSREM, LKVIRK and STDEPAGESA reacted with sera from patients with systemic candidiasis.

The entire human 89kDa heat shock protein (HSP90) gene has been sequenced (Hickey et al, Mol. Cell. Biol., 9, 2615-2626, 1989) and its amino acid sequence deduced and compared to that of heat shock

proteins of other species. Although it appears that the class of heat shock proteins is highly conserved among species, direct comparison and identification of common functional sequences (i.e. epitopes) of the heat shock proteins have not been reported.

It is now found that, notwithstanding the efficacy of the earlier described epitopes, routes to production, other than from the carboxy sequence of the candidal HSP90, can give equal or potentially superior results when used in diagnosis or therapy.

According to the present invention there is provided a functional epitope which is purified from human HSP 90 or which is synthesised to correspond to such a purified epitope, which is, if purified, unchanged or changed by substitution of selected amino acids and if synthesised is identical to a purified epitope or differs from a purified epitope by substitution of selected amino acids, and which cross-reacts with an antibody raised against a stress protein.

The epitope may comprise the amino acid sequence XXXLXVIRKXIV, or XXILXVIXXXXX, wherein X is any amino acid, and may comprise, for example, the amino acid sequence NKILKVIRKNIV.

The epitope may be selected from the amino acid sequences KIRY, NNLGTI, QFIGYPI, KKIK, SKEQV or Candidal equivalent sequence SIKAV, GLELPE or Candidal equivalent sequence FELEES, LDKK or Candidal equivalent sequence LGDQ, WTAN or Candidal equivalent sequence WSAN, NSTMGY or Candidal equivalent sequence TTMSSY, PIVET or Candidal equivalent sequence PIIKE, or KNDK or Candidal equivalent sequence AEDK.

The stress protein may comprise a malarial stress protein, a fungal stress protein or a bacterial stress protein.

The fungal stress protein may comprise a Candidal 92 and/or 47 KDa protein or an Aspergillus 40, 51 and/or 88/84 KDa protein, or a stress protein of Pneumocystis carnii.

The bacterial stress protein may comprise a Coryneform 86 and/or 52KDa protein or a Streptococcal stress protein.

The invention also comprises, in another aspect, a method of making a functional epitope from human HSP 90 or by synthesis, comprising the step of purification from human HSP 90 with or without substitution of selected amino acids or of synthesis of

an epitope which is identical to a purified epitope or which differs from a purified epitope by substitution of selected amino acids, the amino acid subsitution being selected so that the epitope cross-reacts with an antibody raised against a stress protein.

The epitope according to the invention is described as a functional epitope and, as such, has a number of functional uses. In particular it may be used in the diagnosis and treatment of a number of diseases as an alternative and/or improvement to conventional diagnostic and therapeutic methods. The present invention may, for example, be used in the diagnosis and treatment of malaria. This is of topical importance because, as is well known, this disease is fast becoming resistant to current drug treatments, and, as a consequence, is becoming more prevalent throughout the world.

The functional epitope may form the basis of a diagnostic test for malaria, fungal infection, including Pneumocystis carnii or bacterial infection, using an immunological test such as an enzyme-linked immunosorbant assay, a radioimmunoassay or a latex agglutination assay, essentially to determine whether antibodies specific for the epitope, and therefore the particular stress protein, are present in a host

organism. The test may be generally formed by contacting body fluid from the host with an epitope and detecting any complexed material.

In another use, the epitope according to the invention may be employed, using conventional techniques, for screening to obtain activity inhibiting agents for use in the treatment of malaria, fungal and bacterial infections.

In a further use, the epitope according to the invention may be used to generate antibodies, ie. for use as an immunogen, by standard techniques, for example, by injecting any suitable host with the epitope and the serum collected to yield the desired polyclonal anti-epitope antibody after purification and/or concentration. Prior to injection of the host, the epitope may be formulated in a suitable vehicle to provide a composition comprising an epitope together with one or more pharmaceutically acceptable excipients.

Alternatively, the antibodies may be monoclonal in origin and may in general belong to any immunoglobulin class, for example, IgG and/or IgM and/or IgA. The antibody may be of animal, for example, mammalian origin and may be of murine, rat or prefer ably human origin, or may be a murine or rat humanised antibody.

For purification of any anti-epitope antibody, use may be made of affinity chromatography employing an immobilised epitope of the invention as the affinity medium. Such anti-epitope antibodies may be used in both the diagnosis and treatment of fungal and bacterial infections and malaria. As inhibitors of the action of a stress protein, the anti-epitope antibodies may be used either alone or in combination with other pharmaceutical agents, for example, other anti-fungal agents or anti-malarial agents. In addition, such epitopes may be used to produce other inhibitors of fungal or malarial stress proteins, for example, ribosymes and anti-sense RNA will inhibit the translation of stress protein mRNA.

A potential use of such anti-epitope antibodies is in the supportive immunotherapy of, for example, HIV positive patients. Such patients are prone to opportunistic infections due to their immune system being compromised. Examples of such opportunistic infections include Candida, Aspergillus and Pneumocystis carnii. Indeed, sera from HIV positive patients have been shown to be antibody positive to HSP90 of all of these organisms. It is thus proposed to provide such patients with antibody which will recognise HSP90 of these, and other infectous organisms, before these infections become establised and contribute to the death of such patients.

A particularly useful antibody according to the invention is that which recognises the peptide XXXLXVIRKXIV, or XXILXVIXXXXX, wherein X is any amino acid, for example, the peptide NKILKVIRKNIV, and an antibody which recognises one or more of the peptides KIRY, NNLGTI, QFIGYPI, KKIK, SKEQV or Candidal equivalent sequence SIKAV, GLELPE or Candidal equivalent sequence FELEES, LDKK or Candidal equivalent sequence LGDQ, WTAN or Candidal equivalent sequence WSAN, NSTMGY or Candidal equivalent sequence TTMSSY, PIVET or Candidal equivalent sequence PIIKE, or KNDK or Candidal equivalent sequence AEDK.

If desired, mixtures of antibodies may be used for diagnosis or treatment, for example mixtures of two or more antibodies recognising different epitopes of the human stress protein (or Candidal equivalent sequence), and/or mixtures of antibodies of a different class, for example, mixtures of IgG, IgM and IgA antibodies recognising the same or different epitope(s) of a human stress protein (or Candidal equivalent sequence).

The following examples illustrate the invention.

Example 1

Possible immunodominant epitopes of the human HSP 90 have been investigated against sera from patients suffering from various types of diseases in an attempt to provide novel tools for both diagnosis and treatment of disease.

Sera examined included that from patients suffering from systemic candidiasis (47 kDa positive), invasive aspergillosis (88,40 kDa positive), allergic bronchopulmonary aspergillosis, aspergilloma, malaria, Streptococcus faecalis endocarditis, Corneybacterium jeikeium endocarditis and the autoimmune disease systemic lupus erythematosis (SLE).

Epitope mapping of human HSP 90 was carried out against the derived amino acid sequence described in Hickey et al 1989, Mol. Cell. Biol., 9, 2615-2626.

Experimental Details

The 716 amino acid residues were synthesised on polyethylene pins as a complete set of overlapping nonapeptides, in which peptide 1 consisted of residues 1-9, peptide 2 of residues 2-10, etc. Peptide synthesis was performed with Fmoc-protected amino acid esters. The

polyethylene pins themselves were each coupled to Fmoc-ß-alanine. After Fmoc deprotection, the first amino acid was coupled to each pin as dictated by the sequence to be synthesized. Hydrobenzotriazole-mediated coupling reactions were carried out overnight in a N,N-dimethylformamide solution of each side chain protected, Fmoc amino acid. Peptides were synthesized by successive cycles of Fmoc deprotection and addition of one amino acid per pin per day. After completion of the final coupling reaction, and removal of the Fmoc protecting group, the terminal amino group was acetylated in order to remove the unnatural charge of the N terminus of the peptide. Side chain protecting groups were removed by a mixture of trifluoroacetic acid: phenol: ethanedithiol (95: 2.5: 2.5, v/w/v).

The peptides, still coupled to the surface of the pins, were tested against sera by enzyme immunoassay (EIA). Pins were precoated for 1 hr., in microtitre plates containing 1% ovalbumin, 1% bovine serum albumin (BSA) in PBS-T (phosphate-buffered saline, 0.1% Tween 20). They were then incubated at 4°C in patient sera (1/200) washed four times with PBS-T and incubated for 1 h with horseradish-peroxidase conjugated anti-immunoglobulin (1/1000; Sigma, Poole). After further washing, the pins were immersed for 30 min in ABTS (0.5 mg/ml amino-di-3-ethylbenzthiazoline-6-sulphonate in pH

4.0 citrate buffer with 0.03% hydrogen peroxide) and A_{405} measurements made in an EIA plate reader. Pins were cleaned by sonication.

A reaction was considered to be specific if, over at least three wells, the OD was at least > 2-fold above background. The large number of the peptides synthesized effectively acted as negative controls in each test. The mean absorbance of these peptides was low and was used to establish the background level (Geysen et al., 1987).

DETAILS OF PATIENT'S SERA EXAMINED

I. Disseminated Candidiasis

Number	History
1.	leukaemia, neutropenia, blood culture positive *C.albicans*.
2.	post oesophagectomy, blood culture positive, *C.albicans*.
3.	HIV positive, drug abuser, Candidal chorioretinitis.

4. Candidal peritonitis, chronic ambulatory peritoneal dialysis patient, blood culture positive _C.albicans_.

5. Post duodenal resection, positive for _C.albicans_ in two sets of blood culture.

6. Pre (antibody negative), Post antibody positive. Positive for _C.albicans_ in two sets of blood culture. Post cholecystectomy.

7. Post oesophagectomy, chest drain, blood culture positive _C.albicans_.

8. Hepatosplenic candidiasis, yeast seen on biopsy.

9. Chronic ambulatory peritoneal dialysis patient, peritonitis, abdominal abscess, positive for _C. albicans_ in two sets of blood culture.

Antibody profile

Case Number	Candida albicans	Aspergillus fumigatus
1.	M 47, 40 KD G 47, 40 KD	NIL
2.	M 47 KD G 47 KD	88, 84KD trace (M+G combined)
3.	M 47, 48 KD G NIL	NIL
4.	M 47 KD G 47 KD	NIL
5.	M 47 KD G 47 KD	NIL
6.	Pre NIL Post M 40, 47KD, " G 40, 47KD	NIL
7.	M 47 KD G 47 KD	NIL

8.	11/12/91 NIL 17/1/92 M+G 47 KD (positive when combined)	NIL
9.	M 47 KD G 47 KD	88/84KD trace (M+G combined)

Comment <u>C.albicans</u> HSP 90 has bands at 92, 47, 40 KD (as disclosed in GB 2 034 504).

<u>A.fumigatus</u> HSP 90 has bands at 88/84, 51 and 40 KD.

HIV positive patients sera are antibody positive for Candida HSP90, <u>Pneumocystis carnii</u> HSP90 and Aspergillus. Thus, it is proposed to use the antibody in maintenance therapy of such patients, since loss of antibody in these patients may lead to opportunistic infections, such as <u>Pneumocytis carnii</u> and other organisms which produce antigen i.e. HSP90.

II. Invasive aspergillosis

Case Number	History
10.	Fatal, invasive aspergillosis, acute leukaemia
11.	" " " " " " " "
12.	" " " " " " " "
13.	Survivor, " " " " " " "
14.	" " " " " " " "
15.	" " " " " " " "

Antibody Profile

Case Number	Candida albicans	Aspergillus fumigatus
10	NIL	NIL
11	NIL	NIL
12	NIL	M 88,84KD, G 88,84KD
13	NIL	M 88,84KD, G 88,84KD
14	NIL	M 88,84KD, G 88,84KD
15	NIL	M 88,84KD, G 88,84KD

III. Allergic bronchopulmonary aspergillosis

Case Number	C.albicans	Aspergillus fumigatus
16.	NIL	M 88,84KD,
		G 88,84KD
17.	NIL	M 88,84KD,
		G 88,84KD

IV. Aspergilloma

Case Number	C.albicans	Aspergillus fumigatus
18.	NIL	M 88,84KD,
		G 88,84KD

V. Streptococcus faecalis endocarditis

19.	C.albicans	M 47 KD
		G 47 KD
	A.fumigatus	88, 51 KD

The monoclonal antibody described in GB 2 034 504 crossreacts with a S.faecalis immunodominant band at 90 KD as did the rabbit serum raised against this peptide (LKVIRKNIVKKMIE-cys-KLH).

Previous work (Burnie et al, 1985) identified this antigenic band as immunodominant in S.faecalis endocarditis, suggesting that it may form the basis of a test for culture negative endocarditis.

VI. Malaria

	C.albicans	A.fumigatus
20.	M 92 KD	
	G 47 KD	51, 40 KD

A further 5 cases of malaria have been immunoblotted against candidal and aspergillus extracts to show they have antibody which crossreacts with fungal HSP 90.

VII. Corynebacterium jeikeium endocarditis

	C.albicans	A.fumigatus
21.	M 47KD	
	G 47KD	NIL

VIII. Systemic lupus erythematosis (SLE)

	C.albicans	A.fumigatus
22.	G 47KD	NIL
23.	NIL	M 88, 84KD, G 88KD
24.	G 47, 92KD	NIL
25.	M 47KD, G 47KD	NIL
26.	M 40,47KD	
	G 40,47KD	NIL
27.	M 47KD, G 47KD	M 88,84,51,40KD,
		G 88,84,51,40KD
28.	NIL	NIL*

* control serum, so that 6 SLE sera were in the experimental group (see results).

IX. C.guilliermondii meningitis/septicaemia

	C.albicans	A.fumigatus
29.	M 40KD, G 40KD	NIL
	M 47KD, G 47KD (trace)	

RESULTS

EPITOPE MAPPING OF HUMAN HSP 90

Epitope	Human HSP 90 position	Sequence	Recognised by :-
1.	57	KIRY	1/3 invasive aspergillosis 1/1 aspergilloma 4/6 SLE sera
2.	101	NNLGTI	7/9 disseminated candidiasis 47 KDa + (including seroconversion patient *) 1/1 malaria 1/1 _C.guilliermondii_ meningitis

* seroconversion patient - refers to patient 6 whose initial serum was antibody negative, but, on subsequent recovery from the disease, tested antibody positive.

3.	210	QFIGYPI	2/9 disseminated candidiasis 47 kDa + including *seroconverted patient 3/3 invasive aspergillosis (survivors) 1/2 allergic broncho-pulmonary aspergillosis 1/6 SLE sera
4.	271	KKIK	5/9 disseminated candidiasis 47 kDa + including *seroconverted patient 2/3 invasive aspergillosis (survivors) 3/3 invasive aspergillosis (fatal) 1/1 aspergilloma 1/4 SLE sera 1/1 <u>S.faecalis</u> endocarditis

5.	404 KILKVIRK (100% con- served candida)		5/9 disseminated candidiasis including *seroconversion patient 3/3 invasive aspergillosis (survivors) 1/1 invasive aspergillosis (fatal, case no 12) 1/1 malaria
6.	497 SKEQV (Candida S<u>IKA</u>V)		2/9 disseminated candidiasis 3/3 invasive aspergillosis (survivors) 1/3 invasive aspergillosis (fatal) 1/1 aspergilloma 3/6 SLE 1/1 malaria
7.	549 GLELPE (Candida <u>FELEES</u>)		2/9 disseminated candidiasis including *seroconversion patient 3/4 invasive aspergillosis (survivors)

			1/2 allergic bronchopulmonary aspergillosis 1/1 aspergilloma 4/6 SLE 1/1 JK endorcarditis
8.	580 (Candida LGDO)	LDKK	5/9 disseminated candidiasis 1/1 invasive aspergillosis 4/6 SLE 1/1 malaria 1/1 <u>S.faecalis</u> endocarditis
9.	607 (Candida WSAN)	WTAN	2/9 disseminated candidiasis including *seroconversion patient 3/3 invasive aspergillosis (survivors) 1/1 aspergilloma 1/1 malaria 1/1 <u>S.faecalis</u> endocarditis

10.	625 (Candida TTMSSY)	NSTMGY	5/9 disseminated candidiasis including *seroconversion patient 3/3 invasive aspergillosis (survivors) 1/3 invasive aspergillosis (fatal) 1/1 malaria 1/1 <u>S.faecalis</u> endocarditis
11.	642 (Candida PIIKE)	PIVET	2/3 invasive aspergillosis 2/2 allergic bronchopulmonary aspergillosis 3/6 SLE
12.	655 (Candida (AEDK)	KNDK	4/9 disseminated candidiasis including *seroconversion patient 3/3 invasive aspergillosis (survivors) 2/3 invasive aspergillosis (fatal)

2/2 allergic bronchopulmonary aspergillosis
1/1 aspergilloma
5/6 SLE

Conclusion: Epitopes 1,3,4,6,7,8,11 and 12 produced a positive response with sera from SLE patients, i.e. the epitope regognised an autoimmune antibody, and may be classed as autoantibody domains.

Epitopes 2,5,9 and 10 appear to be fungal specific, with epitope 2 being a potential candidal epitope and epitope 9 a potential aspergillosis specific epitope. In addition, epitopes 2,5,9 and 10 may be candidates for potential malarial epitopes, and epitopes 9 and 10, potential S.faecalis epitopes.

Example 2

The peptide LKVIRKNIVKKMIE cys was used to raise the monoclonal sera used in GB 2 034 504. However, the detailed immunogenicity of this peptide is unknown. It was decided to investigate this by replacing each of the amino acids in the above peptide to see the effect, if any, on immunogenicity.

The necessary peptides were first synthesised using conventional techniques and immunogenicity measured using the human sera containing the monoclonal antibody described above. It was also decided to examine a wider epitope, namely NKILKVIRKNIV.

The protocols, and antibody concentrations were the same as those described above in Example 1.

Peptides evaluated

Peptide 1	LKVIRKNIV evaluated for changes in LKVIRK
Peptide 2	NKILKVIRK evaluated for changes in KILK
Peptide 3	LKVIRKNIV evaluated for changes in KNIV

So that in the total sequence the

$\boxed{\text{N K I } \boxed{\text{L K} \text{ V I R } \boxed{\text{K}}} \text{ N I V}}$

Peptide 2 1 3 changes were examined

Sera assayed

1. Monoclonal specific to LKVIRKNIVKKMIE - cys against peptides 1 and 3.

2. Human sera.

Patient Number	Sera	Diagnosis
2	single	Post oesophagectomy, blood culture positive C.albicans.
6	paired[a]	Post cholecystectomy, blood culture positive C.albicans in two sets of blood culture.
30	paired[a]	Neutropenia, leukaemia, blood culture positive C.tropicalis in three sets of blood culture.

31	paired[a]	Neutropenia, leukaemia, blood culture positive C.parapsilosis in two sets of blood culture.
16	paired[a]	Survivor, invasive aspergillosis acute leukaemia.
33	single	P.vivax malaria
34	single	P.falciparum malaria
35	single	P.falciparum malaria
20	single	P.vivax malaria
24	single	SLE

[a] paired means that there is an early serum which was antibody negative which is compared to an antibody positive second serum.

ANTIBODY PROFILES

Patient No.	C.albicans	A.fumigatus
2	M 47KD, G 47KD	M/G 88,84KD (trace) combined
6 Post serum	M 40,47KD, G 40,47KD	NIL
30 Post serum	M 47KD, G 47KD	NIL
31 Post serum	M 47KD, G 47KD	G 40KD
16	NIL	M 88,84KD, G 88,84KD
33	M 47KD, G 47KD	NIL
34	M 92,47KD, G 47KD	G 88KD
35	M 47KD	NIL

20	M 92KD,	
	G 47KD	G 51,40KD
24	G 47KD	NIL

Results:

1. Monoclonal specific to LKVIRKNIVKKMIE - cys

Peptide 1 - LKVIRKNIV

ELISA values measured at 30 minutes, antibody dilution 1:200

Control LKVIRKNIV average ELISA OD = 0.923 (controls)

Substituted amino acid

	L	K	V	I	R	K
			(resultant OD)			
A	0.875	1.102	0.594	0.526	0.111	0.132
C	0.849	0.797	0.527	0.324	0.125	0.119
D	0.909	1.208	0.730	0.195	0.126	0.138
E	1.005	1.183	1.021	0.977	0.141	0.135
F	1.052	0.760	0.512	0.483	0.116	0.108
G	0.054	1.016	0.525	0.161	0.131	0.133
H	0.798	0.926	0.905	0.454	0.107	0.190
I	1.021	0.714	0.491	control	0.117	0.12
K	0.392	control	0.881	0.486	0.092	control
L	control	0.946	0.673	0.659	0.122	0.114
M	0.836	1.075	0.508	0.715	0.118	0.364
N	0.764	1.223	0.769	0.306	0.124	0.137
P	0.77	0.662	0.612	0.489	0.095	0.132

Q	0.656	1.073	0.813	0.602	0.120	0.231
R	0.668	1.032	0.657	0.538	control	0.591
S	0.500	1.026	0.620	0.434	0.121	0.146
T	0.693	0.944	0.740	0.760	0.127	0.139
V	0.788	0.799	control	0.977	0.126	0.124
W	1.022	0.991	1.082	0.895	0.104	0.115
Y	0.584	1.007	1.196	0.788	0.110	0.119
	(L)	(K)	(V)	(I)	(R)	(K)

These results show the importance of the amino acids IRK, with RK being irreplaceable, antibody binding being negligible upon subsitution of these amino acids.

Peptide 3 - LKVIRKNIV

ELISA values measured at 30 minutes, antibody dilution 1:200

Control LKVIRKNIV average ELISA OD = 0.842

	K	N	I	V
A	0.101	0.615	0.109	0.134
C	0.105	0.196	0.122	0.148
D	0.100	0.344	0.100	0.120
G	0.102	0.446	0.101	0.113
F	0.101	0.166	0.142	0.456
G	0.099	0.331	0.100	0.124
H	0.107	0.655	0.114	0.123
I	0.100	0.169	control	0.938
K	control	0.746	0.095	0.136
L	0.103	0.248	0.268	0.831
M	0.099	0.630	0.283	0.430
N	0.101	control	0.103	0.107
P	0.101	0.247	0.107	0.095
Q	0.103	0.596	0.103	0.112
R	0.192	0.819	0.105	0.121
S	0.104	0.942	0.127	0.128
T	0.101	0.691	0.181	0.227
V	0.100	0.210	0.765	control
W	0.098	0.139	0.106	0.123
Y	0.102	0.145	0.107	0.105

These results show the importance of amino acids KNIV.

The total epitope is I R K N I V with the underlined amino acids virtually impossible to replace.

2. Human Sera

All ELISA performed combined IgM and IgG at 30 minutes. Antibody dilution 1:200

Peptide 1

Patient Number		Average ELISA	Number of amino acids giving a reduction to a maximum of 70% control					
			L	K	V	I	R	K
2		1.01	14	4	14	16	5	8
6	Pre	0.1	not applicable					
	Post	0.501	16	9	16	15	4	3
30	Pre	0.22	not applicable					
	Post	0.752	9	0	11	15	2	1
31	Pre	0.1	not applicable					
	Post	0.648	14	0	15	17	4	0
16	Pre	0.347	not applicable					
	Post	0.647	10	2	17	12	2	0
33		1.18	6	1	12	15	2	0
34[a]		0.6	12	0	6	6	0	0
35		1.24	8	2	7	7	2	2
20		1.3	10	1	8	8	1	1
24		0.379	not applicable					

[a] serum examined at 1:1000 dilution.

where ELISA readings were below 0.4, these results were deemed not applicable as they were around the background level.

Peptide 2

Patient Number		Average ELISA	Number of amino acids giving a reduction to a maximum of 70% control			
			K	I	L	K
2		1.37	8	19	19	8
6	Pre	0.10	not applicable			
	Post	0.33	not applicable			
30	Pre	0.22	not applicable			
	Post	0.62	10	18	17	1
31	Pre	0.11	not applicable			
	Post	0.58	10	18	17	3
16	Pre	0.3115	not applicable			
	Post	0.531	2	17	14	13
33			7	16	12	2
34[a]		0.47	4	17	16	2
35		1.36	10	18	17	3
20		1.2	8	16	16	2
24		0.32	not applicable			

[a] serum examined at 1:1000

ELISA values less than 0.4 taken as background, therefore deemed not applicable.

Peptide 3

Patient Number		Average ELISA	Number of amino acids giving a reduction to a maximum of 70% control			
			K	N	I	V
2		0.89	0	0	0	0
6	Pre	0.01	not applicable			
	Post	0.438	0	0	0	0
30	Pre	0.2	not applicable			
	Post	0.52	0	0	3	3
31	Pre	0.35	not applicable			
	Post	0.66	0	1	9	4
16	Pre	0.3	not applicable			
	Post	0.59	3	8	15	11
33		1.03	1	13	11	7
34[a]		0.566	3	15	14	12
35		1.437	0	8	10	3
20		1.18	8	14	15	15
24		0.37	not applicable			

[a] serum examined at 1:1000

ELISA values less than 0.4 taken as background, therefore deemed not applicable.

CONCLUSIONS

The amino acids underlined are vital, i.e. irreplaceable, to the function of the epitope as an immunogen. In addition, sera of patients with different diseases produce antibodies which recognise slightly different amino acids within the same epitope.

Case 24, an SLE patient acted as a positive control, as, although this patients sera was positive for the candidal 47KD protein, it did not recognise any of the 3 peptides of the epitope of the present invention.

Monoclonal epitope		$L\ K\ V\ \underline{I\ R\ K\ N\ I\ V}$
C.albicans epitope	case 2	$N\ K\ \underline{I\ L}\ K\ \underline{V\ I}\ R\ K\ N\ I\ V$
	case 6	$N\ K\ I\ \underline{L}\ K\ \underline{V\ I}\ R\ K\ N\ I\ V$
C.tropicalis	case 30	$N\ \underline{K\ I\ L}\ K\ \underline{V\ I}\ R\ K\ N\ I\ V$
C.parapsilosis	case 31	$N\ \underline{K\ I\ L}\ K\ \underline{V\ I}\ R\ K\ N\ I\ V$
A.fumigatus	case 16	$N\ \underline{K\ I\ L}\ K\ \underline{V\ I}\ R\ K\ N\ \underline{I\ V}$
Malaria: *vivax*	case 33	$N\ K\ \underline{I\ L}\ K\ \underline{V\ I}\ R\ K\ \underline{N\ I}\ V$
	case 20	$N\ K\ \underline{I\ L}\ K\ V\ I\ R\ K\ \underline{N\ I\ V}$
falciparum	case 34	$N\ K\ \underline{I\ L}\ K\ \underline{V\ I}\ R\ K\ \underline{N\ I\ V}$
	case 35	$N\ \underline{K\ I\ L}\ K\ V\ I\ R\ K\ \underline{N\ I}\ V$

The monoclonal described in GB 2 034 504 reacts with an epitope which only represents part of the epitope for yeast infection (C.albicans/C.tropicalis/C.parapsilosis). Infections due to C.tropicalis, A.fumigatus and malaria are more immunoreactive to the KILK epitope. Infections due to malaria and A.fumigatus also recognise an epitope containing NIV.

Thus a more active antibody recognising stress proteins produced in a wider range of diseases than that described in GB 2 034 504 may be produced against the epitope N K I L K V I R K N I V. In addition, antibodies raised against X X X L X V I R K X I V and/or X X I L X V I X X X X X, where X is any amino acid, would also cross react with stress proteins produced in response to fungal and bacterial infection and malaria.

It will be appreciated that it is not intended to limit the invention to the above examples only, many variations, such as might readily occur to one skilled in the art, being possible, without departing from the scope thereof as defined by the appended claims.

CLAIMS

1. A functional epitope which is purified from human HSP 90 or which is synthesised to correspond to such a purified epitope, which is, if purified, unchanged or changed by substitution of selected amino acids and if synthesised is identical to a purified epitope or differs from a purified epitope by substitution of selected amino acids, and which cross-reacts with an antibody raised against a stress protein.

2. An epitope according to claim 1 comprising the amino acid sequence XXXLXVIRKXIV, wherein X is any amino acid.

3. An epitope according to claim 1 comprising the amino acid sequence XXILXVIXXXXX, wherein X is any amino acid.

4. An epitope according to any preceding claim comprising the amino acid sequence NKILKVIRKNIV.

5. An epitope according to claim 1 comprising the amino acid sequence KIRY.

6. An epitope according to claim 1 comprising the amino acid sequence NNLGTI.

7. An epitope according to claim 1 comprising the amino acid sequence QFIGYPI.

8. An epitope according to claim 1 comprising the amino acid sequence KKIK.

9. An epitope according to claim 1 comprising the amino acid sequence SKEQV or Candidal equivalent sequence SIKAV.

10. An epitope according to claim 1 comprising the amino acid sequence GLELPE or Candidal equivalent sequence FELEES.

11. An epitope according to claim 1 comprising the amino acid sequence LDKK or Candidal equivalent sequence LGDQ.

12. An epitope according to claim 1 comprising the amino acid sequence WTAN or Candidal equivalent sequence WSAN.

13. An epitope according to claim 1 comprising the amino acid sequence NSTMGY or Candidal equivalent sequence TTMSSY.

14. An epitope according to claim 1 comprising the amino acid sequence PIVET or Candidal equivalent sequence PIIKE.

15. An epitope according to claim 1 comprising the amino acid sequence KNDK or Candidal equivalent sequence AEDK.

16. An epitope according to any preceding claim, wherein the stress protein comprises a malarial stress protein.

17. An epitope according to any one of claims 1 to 15, wherein the stress protein comprises a fungal stress protein.

18. An epitope according to any one of claims 1 to 15, wherein the stress protein comprises a bacterial stress protein.

19. An epitope according to claim 17, wherein the fungal stress protein comprises a Candidal 92 and/or 47 KDa protein.

20. An epitope according to claim 17, wherein the fungal stress protein comprises an Aspergillus 40, 51 and/or 88/84 KDa protein.

21. An epitope according to claim 17, wherein the fungal stress protein comprises a Pneumocystis carnii protein.

22. An epitope according to claim 18, wherein the bacterial stress protein comprises a Streptoccal 90KD stress protein.

23. An epitope according to claim 18, wherin the bacterial stress protein comprises a Coryneform 86 and/or 52KD stress protein.

24. A method of making a functional epitope from human HSP 90 or by synthesis, comprising the step of purification from human HSP 90 with or without substitution of selected amino acids or of synthesis of an epitope which is identical to a purified epitope or which differs from a purified epitope by substitution of selected amino acids, the amino acid subsitution being selected so that the epitope cross-reacts with an antibody raised against a stress protein.

Patents Act 1977
Examiner's report to the Comptroller under Section 17 (The Search Report)

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Relevant Technical fields

(i) UK Cl (Edition K) C3H (HA3)

(ii) Int Cl (Edition 5) C07K

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NICOLA CURTIS

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASES: WPI, BIOTECH, CAS ONLINE; CHABS

Date of Search

23 DECEMBER 1992

Documents considered relevant following a search in respect of claims 1-24

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
X	EP 0406029 A1 (BURNIE ET AL) see Claim 1	1-4,9-15
X	IMMUNOLOGY, Vol 74, 1991, pages 20-24 Matthews et al "Autoantibody to heat shock protein 90 can mediate protection against systemic candidosis"	1,16-19, 24
X	MOL. CELL. BIOLOGY, Vol 9, No 6, 1989, pages 2615-2626 Hickey et al	1,5-8,10, 12,13,15

Category	Identity of document and relevant passages	Relevant to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

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Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined peri dically in the Official Journal (Patents). The n-line databases considered for search are also listed periodically in the Official Journal (Patents).

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